Investigating Actin and Microtubule Crosstalk through Physical Linkage

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Introduction: Cell motility and directional migration are necessary for cellular functions, while abnormal motility leads to diseases, such as cancer metastasis. Individual cell polarity creating distinct front and back ends needs to be established for cell movement and can be regulated through cytoskeleton components. The cytoskeleton, made of actin, microtubules and intermediate filaments is critical for cell structure and motility. Actin filaments are enriched at the cell leading edge in the form of branched networks in lamellipodia, where they generate force for cell protrusion. Microtubules are distributed throughout the cell and are thought to have a more global role in cell motility by "managing" actin roles in time and space. A leading question of our research is to investigate how microtubules control cell motility. Current models suggest that microtubules deliver "protrusion factors" to the leading edge, are involved in Rho GTPase regulation, and promote disassembly of focal adhesions, yet fail to show a direct link between microtubules and cell protrusion. Electron microscopy images have shown association of branched actin filaments with microtubules in neurons, suggesting that microtubules initiate assembly of these branching networks[3]. This research aims to provide evidence for microtubule associated branched actin at the cell leading edge of non-neuronal cells, and thus for a direct mechanism of microtubule controlled cell protrusion through branched actin formation at the microtubule tip.

Materials and Methods: COS-7 cells were cultured in DMEM containing 10% FBS. Latrunculin B (LatB) in DMSO, an actin depolymerizing agent, was used to clear excess actin and allow for visualization of microtubule tips at the dense branched actin areas. Cells were treated with DMSO as a control, 2.5µM LatB, or 5µM LatB for one hour. Samples for confocal microscopy were fixed and stained with fluorescent phalloidin to detect actin filaments and α-tubulin antibody to reveal microtubules. Samples for platinum replica electron microscopy (PREM) were processed according to protocol and imaged using transmission electron microscopy[4].

Results and Discussion: DMSO treated control cells in Fig.1A were well-spread and contained microtubules and actin filaments distributed throughout the cell. Cells treated with 2.5µM or 5µM LatB contracted after treatment and exhibited processes extending outward from cell bodies. Fig.1B shows actin (orange) at microtubule tips (green) after 2.5µM LatB treatment for 1 hour. Increasing the concentration of LatB to 5µM for 1 hour produced greater contracted cells with longer processes. Fig.1C shows PREM imaging of a 2.5µM LatB treated cell showing branched actin filaments along microtubules. Branched actin networks were densely concentrated making them difficult for visualization under PREM imaging. Fig1D shows branched actin network formation observed at a microtubule tip in a cell treated with 5µM.

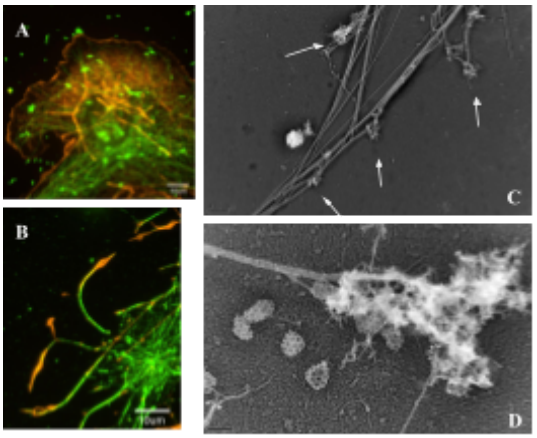

Figure 1. (A) Confocal image of DMSO treated COS-7 cell fixed with 0.2% GA (B) Confocal image of 2.5 µM Lat B treated cell fixed with 4% PFA showing branched actin (orange) at microtubule tips (green) (C) PREM image of 2.5 µM Lat B treated cell showing branched actin formation along microtubules (D) PREM image of a branched actin network formed at a microtubule tip in cell treated with 5µM Lat B.

Conclusions: In this study we demonstrate that a subpopulation of microtubules was associated with branched actin network at their tips at the cell periphery, which likely leads to microtubule-controlled leading edge protrusion. This work provides a new model for investigation of direct microtubule involvement in cell motility and migration. Further work may provide evidence of a linker physically joining actin and microtubules.

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References: [3] Nadia Efimova et al. Branched actin networks are assembled on microtubules by adenomatous polyposis coli for targeted membrane protrusion. *J Cell Biol* 7 September 2020 [4] Svitkina T. Imaging Cytoskeleton Components by Electron Microscopy. Methods Mol Biol. 2016